Metabolic Studies on Isolated Hair Follicles: Hair Follicles Engage in Aerobic Glycolysis and Do Not Demonstrate the Glucose Fatty Acid Cycle

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The matrix cells of the hair follicle have one of the highest rates of cell division in the mammalian body, but their fuel metabolism is poorly understood, due mainly to the difficulty in obtaining viable intact follicles from the skin. We have previously shown that viable and intact rat hair follicles can be isolated by shearing, and in this study we now report on their fuel metabolism. In this study we have shown that the hair follicle exhibits aerobic glycolysis, in that the total glucose utilized by the hair follicle, only 10% is oxidized to CO₂. We have also shown that, in the absence of glucose, the hair follicle is capable of utilizing other fuels such as palmitate and β-hydroxybutyrate. However, neither palmitate or β-hydroxybutyrate had any effect on the rate of glucose utilization or on [U-¹⁴C] glucose oxidation, showing that glucose sparing via the glucose fatty acid cycle does not operate in the hair follicle. Measurements of glucose flux through the pentose phosphate pathway accounted for only 3% of the total glucose utilized by the hair follicle, although this value represented 32% of the total glucose oxidized. Both palmitate and β-hydroxybutyrate inhibited glucose flux through the pentose phosphate pathway. J Invest Dermatol 96:875–879, 1991

Adachi and Uno [1] have shown that plucked human hair follicles preferentially metabolize glucose to lactate rather than to CO₂. This is not necessarily surprising, as hair follicles are derived from epidermal downgrowths [2], and skin is one of those tissues that Krebs [3] described as engaging in aerobic glycolysis, which is the preferential metabolism of glucose to lactate despite the presence of oxygen. However, the findings of Adachi and Uno [1] must be considered incomplete for two reasons: first, they isolated hair follicles by plucking, and this technique generally leaves the dermal papilla and a large part of the hair follicle bulb behind in the skin [4], yet the dermal papilla regulates hair growth, and the matrix cells at the base of the bulb are essential for this growth [5]. Secondly, plucking causes a great deal of cell damage by rupturing the hair matrix from the follicle [6] and it is known that damaged tissues demonstrate aerobic glycolysis [7]. It is important therefore to study the metabolism of hair follicles that have been isolated whole and without damage.

We have previously shown that the technique of shearing isolates whole, undamaged hair follicles [8]. Recently [9], we have shown that hair follicles isolated by shearing are viable, as judged by light and electron microscopy, [methyl ³H] thymidine uptake into DNA, [U-¹⁴C] leucine uptake into protein, [³⁵S] methionine uptake into keratin, and by tritiated thymidine autoradiography. We now report on the fuel metabolism of freshly isolated rat hair follicles isolated by shearing.

MATERIALS AND METHODS

Materials ATP and NADH luciferase monitoring kits were supplied by LKB Instruments Ltd. Adenine nucleotides, NAD⁺, palmitate, de-fatted bovine serum albumin, pyruvate kinase, adenylate kinase, amyloglucosidase, and glucose dehydrogenase came from BCL. Hyamine hydroxide came from BDH. All radionucleotides were supplied by Amersham. All other chemicals came from Sigma.

Isolation of Rat Hair Follicles Follicles were isolated from dorsal skin, taken from 8- to 12-day-old rats by shearing as previously described [8].

Adenine Nucleotides These were measured using the methods based on the luciferase assay of ATP [10,11]. Hair follicles were placed in 500 μl of 5% perchloric acid (PCA) and left on ice for 30 min. At the end of this time 20 μl of supernatant was removed and neutralized with 20 μl of 1 M KOH. For the determination of ATP, 10 μl of sample was incubated with 10 μl of reaction buffer (0.3 mM phosphoenol pyruvate, 9 mM MgCl₂, 5 mM KCl, 0.1 M Tris-acetate, pH 7.75). For the determination of ATP + ADP, the reaction contained 20 U ml⁻¹ pyruvate kinase and for ATP + ADP + AMP 20 U ml⁻¹ pyruvate kinase and 150 U ml⁻¹ adenylate kinase. Samples were incubated for 30 min at 37°C, after which 80 μl of 0.1 M Tris-acetate pH 7.75 were added and the samples assayed for ATP content. ATP was stable in 5% PCA for at least 3 h.
within which time assays were carried out. Standard curves were linear over the range 0 to 100 pmoles ATP.

**Glycogen Content** This was performed using a NADH luciferase linked assay [12]. Isolated hair follicles were placed in 20 μl of 5% PCA and left on ice for 30 min, following which 13 μl of 1 M KHCO₃ and 67 μl of 0.2 M sodium acetate pH 4.8 containing 14 U ml⁻¹ of amyloglucosidase were added and the tubes incubated for 2 h at 37°C in a shaking water bath. The samples were then centrifuged at 12,000 × g for 15 min, at room temperature. Twenty microliters of supernatant was removed and glucose measured by glucose dehydrogenase as follows. To the 20-μl aliquots were added 380 μl of 0.1 M potassium phosphate, 100 μM NAD⁺, 0.4 units of glucose dehydrogenase, pH 7.75. Samples were then incubated for 30 min at 37°C, and 200-μl aliquots removed for NADP determination using LKB NADH luciferase assay kits. Standard curves were linear over the range 0 to 100 pmoles glucose. Because glycogen is of indeterminate molecular weight, it was reported in glucose units.

**Determination of Glycolytic Rate** This was made by measuring the rate of release of tritium from carbon 2 during the conversion of [2-²H] glucose to fructose-6-phosphate [13]. Twenty isolated hair follicles were placed in 500 μl of bicarbonate-buffered medium containing 5 mM [2-²H] glucose (SA 0.8 Ci/mol) and incubated at 37°C in an atmosphere of 5% O₂/95% CO₂ in a shaking water bath. Rates of H₂O release were measured over a time course of 1, 3, and 6 h as follows. After incubation, 10 μl of sample medium was removed and placed in a small plastic (LP) tube to which was added 10 μl of 1 M HCl. The tube was then placed in a stoppered scintillation vial containing 0.5 ml H₂O in an incubator overnight at 37°C, and the H₂O recovered in scintillation vials was measured by liquid scintillation spectrometry. With each experiment controls were carried out in which known amounts of H₂O were acidified with HCl. The percentage recovery was then calculated and the results corrected for this.

**D-[U-¹⁴C] Glucose, [U-¹⁴C] Palmitic Acid, and D-3 Hydroxy [3-¹⁴C] Butyrate Oxidation** These were measured by incubating hair follicles in 500 μl of bicarbonate-buffered medium containing either 5 mM D-[U-¹⁴C] glucose (SA 0.2 Ci/mol), 0.5mM [U-¹⁴C] palmitate/0.75% bovine serum albumin (SA 1.0 Ci/mol) or 1 mM D-3 hydroxy [3-¹⁴C] butyrate (SA 2.0 Ci/mol). Measurements were made over a time course of 1, 3, and 6 h in plastic tubes placed in scintillation vials in a shaking water bath at 37°C in an atmosphere of 95%O₂/5% CO₂. After incubation, 0.5 ml of hyamine hydroxide was injected into the scintillation vials previously described [15], substrate oxidation was arrested, and CO₂ liberated into the hyamine hydroxide by injecting 100 μl of 25% PCA into the plastic tube.

**Glucose Metabolism by the Pentose Cycle Pathway** The contribution of the pentose cycle pathway to glucose metabolism by the hair follicle was investigated by using the methods established by Katz and Wood [16]. Hair follicles were incubated in 500 μl of bicarbonate-buffered medium containing either 5 mM [1-¹⁴C] glucose (SA 0.2 Ci/mol) or 5 mM [6-¹⁴C] glucose (SA 0.2 Ci/mol). The rates of glucose oxidation were measured as described above. The contribution of the pentose cycle pathway to hair follicle glucose metabolism was then calculated using the following equation:

\[
G_1CO_2 + G_6CO_2 + 2G_1CO_2 = 3P + 1 + 2P,
\]

where G₁CO₂ and G₆CO₂ are the specific yields of ¹⁴CO₂ from [1-¹⁴C] and [6-¹⁴C] glucose, respectively, and P is the fraction of glucose metabolized by the pentose cycle pathway. The absolute rate of glucose metabolized by the pathway was then calculated as glucose utilized × P.

**Effects of Palmitate and B-Hydroxybutyrate on Glucose Utilization and Oxidation** In order to investigate the effects of palmitate and b-hydroxybutyrate on glucose utilization and oxidation, we carried out experiments, as described above. Rates of [2-²H] glucose utilization and [U-¹⁴C] oxidation were measured in the presence of either 0.5 mM palmitate or 1 mM b-hydroxybutyrate.

**Results**

**Adenine Nucleotides and Glycogen Content** The ATP, ADP, and AMP contents of freshly isolated hair follicles were 9.40 ± 0.65, 6.45 ± 0.47, and 1.35 ± 0.08 pmol per hair follicle, respectively (mean ± SEM, n = 15 rats, 20 follicles in duplicate per rat). From these values an ATP/AMP ratio of 7.0 was obtained and an energy charge of 0.74 by using the equation:

\[
E = \frac{[ATP] + 0.5[ADP]}{[ATP] + [ADP] + [AMP]},
\]

where E is the energy charge [17].

The glycogen content of freshly isolated hair follicles was 718 ± 116 pmol per hair follicle (mean ± SEM). Observations were made on hair follicles from 8-, 9-, and 10-day-old rats by using 20 follicles in duplicate from four rats at each age (n = 12 rats, total 20 follicles in duplicate per rat).

**Metabolic Studies** The results of this study are shown in Table I. Rates of glucose oxidation and glucose utilization were linear over the course of these experiments as shown in Figs 1 and 2, respectively. The overall rate of glucose utilization as determined by the flux through glucose phosphate isomerase was 152 ± 17.5 pmol glucose per follicle/h, and the rate of glucose oxidation 15.0 ± 3.0 pmol glucose/follicle/h. Therefore, of the total amount of glucose utilized by the hair follicle, only 10% is oxidized to CO₂. The glycogen content of hair follicles after 6 h incubation in bicarbonate-buffered medium containing 5 mM glucose was 555 ± 118 pmol glycogen/follicle, and did not differ significantly from freshly isolated hair follicles. The ATP content of hair follicles after 6 h incubation did not differ significantly from fresh follicles, indicating that they retain their metabolic viability (Table I).

**Table I. Fuel Metabolism by Freshly Isolated Rat Hair Follicles**

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<tr>
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<th>5 mM [2-²H] Glucose Hydrolysis</th>
<th>5 mM [U-¹⁴C] Glucose Oxidation</th>
<th>0.5 mM [U-¹⁴C] Palmitate Oxidation</th>
<th>1 mM D-[3-¹⁴C] β-Hydroxybutyrate Oxidation</th>
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<tbody>
<tr>
<td>Substrate metabolized (pmol/follicle/h [mean ± SEM])</td>
<td>152 ± 17.5</td>
<td>15.0 ± 3.0</td>
<td>4.35 ± 0.4</td>
<td>8.1 ± 0.8</td>
</tr>
<tr>
<td>Glycogen content of follicles after incubation (pmol/follicle [mean ± SEM])</td>
<td>555 ± 118</td>
<td>662 ± 39</td>
<td>502 ± 145</td>
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<tr>
<td>ATP content of follicles after incubation (pmol/follicle [mean ± SEM])</td>
<td>8.0 ± 1.6</td>
<td>9.8 ± 1.6</td>
<td>7.0 ± 1.3</td>
<td>5.4 ± 1.6</td>
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* Hair follicles were isolated by shearing, and fuel metabolism, glycogen contents, and adenine nucleotides determined as described in Materials and Methods. All dynamic measurements were made over a time course of 1, 3, and 6 h, during which rates of hydrolysis and oxidation were linear. Results are expressed as the rate of substrate hydrolysis or oxidation (mean ± SEM) for five separate experiments (five rats) with 20 hair follicles in duplicate from each rat at each time point. The glycogen and ATP contents were determined after 6-h incubation, and there were no significant changes from their contents in freshly isolated follicles, which were 718 ± 116 pmol (mean ± SEM, n = 12) and 9.40 ± 0.65 (mean ± SEM, n = 15), respectively.
The rates of palmitate and β-hydroxybutyrate oxidation were also linear over the course of the experiments as shown in Figs 3 and 4, respectively. Table I shows that the rate of palmitate oxidation was 4.35 ± 0.42 pmoles palmitate/follicle/h and the rate of β-hydroxybutyrate oxidation was 8.10 ± 0.85 pmoles β-hydroxybutyrate/follicle/h. The glycogen content of hair follicles after 6 h incubation in bicarbonate-buffered medium containing 0.5 mM palmitate or 1.0 mM β-hydroxybutyrate were 662 ± 39 and 502 ± 145 pmoles glycogen/follicle, respectively; these values did not differ significantly from fresh follicles. The ATP contents after 6-h incubations were 7.0 ± 1.3 and 5.4 ± 1.6 pmoles ATP per follicle for palmitate and β-hydroxybutyrate, respectively; these showed no significant difference from fresh follicles.

Having shown that rat hair follicles were capable of utilizing a number of potential fuels, we decided to further explore hair follicle glucose metabolism by measuring the rates of glucose flux through the pentose phosphate pathway, and to study the effect of palmitate and β-hydroxybutyrate on hair follicle glucose metabolism, including their effects on the glucose flux through the pentose phosphate pathway.

The results of this study are shown in Table II. The contribution of the pentose cycle to glucose metabolism in the hair follicle was small, accounting for only 3% of the total glucose metabolized with a value of 4.8 pmoles of glucose/follicle/h. However, this value represents 32% of the total glucose oxidized.

Table II also shows the results of experiments carried out to investigate the effects of palmitate and β-hydroxybutyrate on hair follicle glucose metabolism. The rates of glucose utilization and oxidation in the presence of 0.5 mM palmitate were 259 ± 56 pmoles/follicle/h and 13.50 ± 6.1 pmoles/follicle/h, respectively; these did not differ significantly from the values observed for glucose utilization and oxidation in the absence of palmitate. Similarly, the rates of glucose utilization and oxidation in the presence of...
for kidney, 4.0 for epididymal fat pad, 49.8 for perfused heart, and 124 for thigh muscle. This finding would appear to be consistent with the proposed role of AMP and the adenylyl kinase reaction in the regulation of glycolysis. In tissues where there is a large variation in the rate of energy utilization, as in the muscle, the ATP/AMP ratio is high, whereas in tissues where there is a small variation in the rates of energy utilization the ratio is lower [19].

The studies on glucose metabolism by isolated rat hair follicles show that, of the total amount of glucose utilized by the hair follicle (152 ± 17.5 pmol follicle/h), only 10% is oxidized to CO₂ (15.0 ± 3.0 pmol follicle/h). The remaining 90% of [2-³H] glucose hydrolysis can be attributed to lactate production. This attribution is strengthened by our earlier studies on the eccrine sweat gland [12,25], where we have shown that the direct measurement of lactate production in vitro gives a comparable finding to that calculated from determining the differences between glycolysis and glucose oxidation [12].

The rat hair follicle therefore oxidizes only 10% of its glucose. This compares to a figure in the working heart, for example, of 59% [20]. Thus the hair follicle can be described as a tissue that engages in aerobic glycolysis [3], which shows that the findings of Adachi and Uno [1] can be attributed neither to the damage caused by plucking, nor to the incomplete recovery of the bulb that plucking affects.

Our metabolic studies on the hair follicle have also shown that the hair follicle is capable of metabolizing palmitate and β-hydroxybutyrate, which is the first time that such observations have been made on hair follicles. However, it cannot be calculated that palmitate oxidation in the hair follicle will yield 561 pmol of ATP/follicle/h, and β-hydroxybutyrate oxidation 243 pmol ATP/follicle/h. These can be compared with glucose utilization that yields 814 pmol ATP/follicle/h. Thus, although our ATP measurements indicate that fats will maintain hair follicle viability, it appears that they are poor hair follicle fuels.

Experiments carried out to investigate the effects of palmitate and β-hydroxybutyrate on glucose utilization and oxidation showed that they had no significant effect on either glucose utilization or oxidation and we therefore conclude that the glucose-fatty acid cycle [21] does not play an important role in the metabolism of the hair follicle. This observation complements that of Welch et al [12] who reported the absence of the glucose fatty acid cycle in the eccrine sweat gland, another tissue that preferentially metabolizes glucose over fats. The brain, which also primarily metabolizes glucose, does not exhibit a glucose fatty acid cycle either [22], and this may be a feature of tissues whose major fuel is glucose rather than fats.

Our studies on isolated hair follicles have also shown that the pentose phosphate pathway operates in the hair follicle. This observation complements that of previous workers who have shown that the enzymes required for a functional pentose phosphate pathway are present in the hair follicle [1]. In this study we have found the contribution of this cycle to the overall glucose metabolism of the follicle is small (3%). This value is similar to the 2.5% reported for plucked human hair follicles [1], but less than that found in 4-day-old rat epidermis, in which values of 10% have been reported [23].

1 mM β-hydroxybutyrate, which were 101 ± 25 pmol follicle/h and 5.17 ± 1.5 pmol follicle/h, respectively, did not differ significantly from glucose alone.

The presence of 0.5 mM palmitate or 1 mM β-hydroxybutyrate did, however, result in a significant decrease in the amount of glucose metabolized by the pentose cycle (Table II) to 2.8 pmol follicle/h in the presence of palmitate (p < 0.001), and 2.5 pmol follicle/h in the presence of β-hydroxybutyrate (p < 0.001).

**DISCUSSION**

The adenine nucleotide contents of freshly isolated rat hair follicles were used to calculate an energy charge value of 0.74 and an ATP/AMP ratio of 7.0. These values compare well with those from other rat tissues [18] where the ATP/AMP ratios were 10.5 for liver, 6.8

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**Table II. Effects of Palmitate and β-Hydroxybutyrate on Hair Follicle Glucose Metabolism**

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<tbody>
<tr>
<td>5 mM glucose</td>
<td>142 ± 60</td>
<td>9.3 ± 2.2</td>
<td>19.8 ± 2.0</td>
<td>7.0 ± 2.0</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>5 mM glucose + 0.5 mM palmitate</td>
<td>259 ± 56</td>
<td>13.5 ± 6.1</td>
<td>19.0 ± 4.2</td>
<td>10.7 ± 3.5</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>5 mM glucose + 1 mM β-hydroxybutyrate</td>
<td>101 ± 25</td>
<td>5.1 ± 1.5</td>
<td>12.0 ± 3.5</td>
<td>5.1 ± 1.5</td>
<td>2.5 ± 0.1</td>
</tr>
</tbody>
</table>

*Experiments were carried out as described in the text, freshly isolated hair follicles were incubated for 3 h as described in the text, previous experiments having shown that the rates of glucose hydrolysis and oxidation were linear for up to 6 h. Results are expressed as the mean ± SEM for five separate experiments (five rats) with 20 hair follicles in duplicate from each rat. Neither 0.5 mM palmitate nor 1 mM β-hydroxybutyrate had a significant effect on either the rate of glucose hydrolysis or the overall rate of oxidation; however, they both significantly reduced the rate of glucose oxidation via the pentose phosphate pathway.

*The rate was significantly different (p < 0.05) from the rate with 5 mM glucose alone when analyzed by Student t test.
Although we have shown that the contribution of the pentose phosphate pathway to the overall glucose metabolism in isolated rat hair follicles is small, this value does account for 32% of the total glucose oxidized by the hair follicle, and is, moreover, significantly inhibited by both palmitate and β-hydroxybutyrate.

The major activity of the hair follicle is matrix cell division, leading to hair growth. The pentose phosphate pathway will, therefore, be generating precursors for DNA synthesis and also NADPH for lipid synthesis. It is unlikely that fats would be inhibiting DNA synthesis, but their inhibition of the pentose phosphate pathway may perhaps be mediated via NADPH. Fatty acyl CoA inhibits acetyl CoA carboxylase [24], which will reduce NADPH consumption and so inhibit the pentose phosphate pathway.

In conclusion, therefore, we have characterized some aspects of the fuel metabolism of the hair follicle and have shown that it engages in the aerobic glycolysis. In this, it appears to be typical of the skin. The metabolism of two different skin organs, the epidermis [25] and the eccrine sweat gland [26], have been characterized; each in vitro engages in aerobic glycolysis [25,26]. As the hair follicle is derived, embryologically, from epidermal downgrowth [2], it is not surprising that the hair follicle shares in the skin’s aerobic glycolysis.

The teology of aerobic glycolysis in the hair follicle remains puzzling as the matrix cells of the hair follicle contain many mitochondria. Recently, Newsholme and his associates have made an interesting hypothesis [27]. They have noted that many of the mitochondria-containing tissues that express aerobic glycolysis—lymphocytes, endothelial cells, the skin, cancer cells—possess great potential for cell division; Weinstein and Mooney [28] have noted that the matrix cells of the hair follicle have one of the fastest rates of cell division in the mammalian body. Newsholme and his colleagues [27] have proposed that their major fuel might actually be glutamine, whose metabolism would generate nitrogen for purine and pyrimidine synthesis. It will be important in the future, therefore, to study glutamine metabolism in the hair follicle, so as to further characterize skin metabolism and aerobic glycolysis in mitochondria-containing tissues.

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REFERENCES